IN THE UNITED STATES PATENT AND TRADEMARK OFFICE	
INVENTORS: Jian Ling, et al. SERIAL NO.: FILED: TITLE: Methodology of Using Raman Imaging Microscopy for Evaluating Drug Action Within Living Cells	ATTY DKT NO.: P-17.111(CON) EXAMINER: LAW OFFICE NO.:

TO: COMMISSIONER FOR PATENTS
Mail Stop: RESPONSE
P.O. Box 1450
Alexandria, VA 22313-1450

JOINT AFFIDAVIT OF DR. JIAN LING AND MICHAEL A. MILLER

We, Dr. Jian Ling and Michael A. Miller, being duly sworn, depose and state as follows:

1. We are both employed by Southwest Research Institute, Assignee of the above identified patent application. Resumes for each of us are attached hereto as Exhibits A and B.

2. We have both been extensively involved in the use of Raman imaging to visualize drug uptake at the cellular level, Dr. Ling, a Senior Research Engineer, and Mr. Miller as a manager - Research & Development and physical chemist. It was from this extensive work in Raman imaging that patent application Serial No. 09/804,774, was filed on March 13, 2001, from which this application is a continuation.

3. We have both reviewed the entire file wrapper for patent application Serial No. 09/804,774, filed on March 13, 2001, (hereinafter the "Parent Application").

4. We both participated in an interview with Examiner Deborah Davis and her Supervising Examiner for the Parent Application on October 28, 2003. After the interview, we assisted and gave comments for the preparation of the "Amendment After Final," which was filed in the Parent Application.

5. Thereafter, we reviewed the Advisory Action dated December 2, 2003, received from Examiner Davis and her comments therein, including the following:

> In response to applicant's argument that the Grow reference does not apply because it does not address "imaging" is not found persuasive. The use of imaging is addressed in column 22, lines 43-67, column 56, lines 1-20, claims 5 and 15. Although the Grow reference does not address drug interaction in living cells, the Sharonov, et al. reference teaches the advantage of this limitation (see final office action).

6. This Affidavit is in rebuttal to the above quoted comments of Examiner Davis.

7. The primary reference of Grow (U.S. Patent No. 5,866,430) uses bio-concentration to obtain increased sensitivity of the Raman spectrum. The Grow reference relies to a large extent upon binding interactions in a bio-concentrate. The Grow reference is a portable biosensor device using Raman spectroscopy.

8. The Grow reference deals with the analyte identification, concentration and (in some cases) quantification using Raman spectroscopy.

9. In the "Amendment After Final," Applicants indicated the Grow reference did "not [use] direct Raman imaging." [Emphasis added.] This statement is true.

10. What the Grow reference does is combine a bio-concentrator with another compound and the bio-concentrator is detected. Referring to the portion of the specification of Grow, which Examiner Davis referenced, it states therein the following:

> In each case, the Raman spectrometer system or subsystem receives as its input the emitted radiation from the bioconcentrator or bioconcentrator-analyte complex or biological... [Emphasis added.]
> See column 22, lines 53-56.

11. This is emphasized again in the portion of the Grow specification also referenced by Examiner Davis, which states the following:

> In yet another example of the invention, a series of different categories of bioconcentrators that are specific for different chemical and biological warfare agents may be immobilized on the surface of a dipstick... The dipstick may then be inserted into samples collected by a bioaerosol liquid impactor, rinsed, and inserted into an imaging Raman microprobe. Anthrax spores captured by the antibodies...bound to the acetylcholine receptor... can be detected and identified simultaneously by the imaging Raman system. [Emphasis added.]

12. Again, in the portion quoted by Examiner Davis, a close examination indicates it is the bio-concentrator that is being shown by Raman imaging, not the particular drug under investigation.

13. Even turning to Claims 5 and 15, referenced by Examiner Davis for the "imaging spectrometer" as stated therein, the imaging spectrometer is detecting the bio-concentrator, which is bound to an analyte. It is the bio-concentrator, not the analyte, that the imaging spectrometer is detecting.

14. While it may have been an overstatement in the "Amendment After Final" to state "the Grow reference does not use Raman imaging," it is not an overstatement to indicate the Grow reference does not (a) evaluate drug action within living cells, and (b) use direct Raman imaging. The imaging as is done in Grow is used to detect the bio-concentrated mixture, not imaged drug action in living cells.

15. The Sharonov reference demonstrates the use of fluorescence imaging to determine distribution of anti-tumor drugs within living cancer cells, not the use of direct Raman imaging. It is important to state that fluorescence and Raman imaging are based upon two fundamentally different and independent physical processes, which can be delineated by the field of quantum mechanics. Fluorescence is a process in which electrons are promoted to an excited electronic state and then decay radiatively to an electronic state of lower energy than the excited state, whereas Raman is a process in which photons are scattered by means of exciting electrons to, and returning from, virtual states with net energy difference equivalent to a vibrational state. In addition,

Sharonov does not disclose the various steps as contained in either independent Claim 1 or independent Claim 15. There is no "processing," dividing," or "comparing" to determine drug distribution within living cells as is specifically claimed.

16. We have both been working in the field of Raman spectroscopy for a number of years. We are familiar with the scientific publications and journals in this area. Based on our knowledge, none of the publications or journals we know about has ever suggested the use of direct Raman imaging to evaluate drug action in living cells, which would include uptake, distribution, local bonding, cellular resistance, pharmcokinetics, and metabolism of a drug . This is true from the time after the Sharonov reference was first published in 1994, up until the date of filing of the parent application.

Further, Affiants sayeth not.

Dr. Jian Ling

Michael A. Miller

STATE OF TEXAS §
§
COUNTY OF BEXAR §

BEFORE ME, the undersigned authority, on this day personally appeared DR. JIAN LING, known to me to be the person of that name in the capacity so indicated, who signed the foregoing instrument, and acknowledged the same to be his free act and deed.

GIVEN under my hand and seal of office this 29th day of December, 2003.

Shirley E. McDonald
Notary Public

Shirley E. McDonald
Printed Name of Notary

Commission Expires April 5, 2005

STATE OF TEXAS §
§
COUNTY OF BEXAR §

BEFORE ME, the undersigned authority, on this day personally appeared MICHAEL A. MILLER, known to me to be the person of that name in the capacity so indicated, who signed the foregoing instrument, and acknowledged the same to be his free act and deed.

GIVEN under my hand and seal of office this 29th day of December, 2003.

Shirley E. McDonald
Notary Public

Shirley E. McDonald
Printed Name of Notary

Commission Expires April 5, 2005

CERTIFICATE OF MAILING

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited on the date shown below with the United States Postal Service in an envelope addressed to the "Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450," as follows:

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Date: _____, 2003	Date: December 31, 2003

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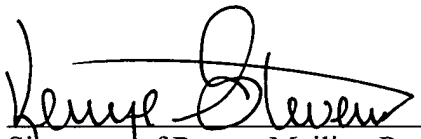

Signature of Person Mailing Paper or Fee

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CURRICULUM VITAE

Name: **Jian Ling, Ph.D.**

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Research Interests: Optical technologies for drug research and disease diagnosis, molecular spectroscopy and imaging using fluorescence, Raman technologies, biological microscopy, biomedical signal processing and image processing.

Education:

Jan. 1998 - Dec. 2001 The University of Texas at Austin, Biomedical Engineering Program. Austin, Texas. **Ph.D.** of Biomedical Engineering. G.P.A. 3.75 (A = 4.0).

Sept. 1995 - Dec. 1997 The University of Texas at San Antonio, Department of Electrical Engineering. San Antonio, Texas. Graduate Courses; G.P.A. 4.0 (A=4.0).

Sept. 1990 - May 1993 The University of Houston, Department of Electrical Engineering. Houston, Texas. **Master** of Science in Electrical Engineering. G.P.A. 3.67 (A=4.0).

Jan. 1990 - Sept. 1990 Sam Houston State University, Department of Physics. Huntsville, Texas. Enrolled in graduate program in Physics.

Sept. 1986 - July 1988 East China Normal University, Department of Electrical Engineering. Shanghai, China. Enrolled in graduated program in Electrical Engineering.

Professional Experiences:

June 1997 - Present — Senior Research Engineer, Bioengineering Department, Southwest Research Institute, San Antonio, Texas.
Duties include algorithm design, signal processing and image processing for biomedical research and development. Lead in the development of Raman imaging microscopy for biomedical applications and drug research.

June 1994 - June 1997 — Research Engineer, Dept. of Biosciences and Bioengineering, Southwest Research Institute, San Antonio, Texas.
Duties include software development and algorithm development for several medical devices. Examples are an oximetry and cardiac output monitoring system, a cardiovascular monitoring and diagnostic device, a pacemaker system, and a trauma patient simulation model.

Feb. 1993 - June 1994 — Research Assistant, Dept. of Surgery, Baylor College of Medicine, Houston, Texas.
Duties include the evaluation of a reflectance oximetry sensor in surgical environment, assessment of an oscillometric and tonometric blood pressure monitoring system on adults, children and neonates, designing of a microprocessor controlled artificial heart system.

Jan. 1992 - May 1993 — Teaching Assistant, Dept. of Electrical Engineering, University of Houston, Houston, Texas.
Duties include conducting lab experiment and evaluating student assignment.

June 1990 - Aug. 1990 — Research Assistant, Texas Accelerator Center, The Woodlands, Texas.
Duties include computer simulation of an ion source.

Jan. 1990 - May. 1990 — Teaching Assistant, Dept. of Physics, Sam Houston State University, Huntsville, Texas.
Duties include conducting lab experiment and evaluating student assignment.

Membership:

1. IEEE Signal Processing Society member.

2. IEEE Engineering in Medicine and Biology Society (EMBS) member.

Publications:

A. Master Thesis: "A comparative automated study of the EEG in human sleep stage 1 and sleep stage 1-REM ", May, 1993.

B. Ph.D. Dissertation: "The Development of Raman Imaging Microscopy to Visualize Drug Actions in Living Cells", December, 2001.

C. Peer Review Journal Publications:

1. **Ling J.**, Ohara Y., Orime Y., Noon G.P., Takatani S. "Clinical evaluation of a new oscillometric blood pressure monitoring system in adults and children based on the 1992 AAMI SP-10 Standards", *Journal of Clinical Monitoring*, 11(2), 1995.

2. Takatani S., **Ling J.**, "Optical oximetry sensors for whole blood and tissue", *IEEE Engineering in Medicine and Biology Magazine*, 13(3), 1994.

3. Takatani S., Orime Y., Tasai K., Ohara Y., Naito K., Mizuguchi K., Makinouchi K., **Ling J.**, Noon G.P., Nose Y., "Totally implantable TAH and VAD with multipurpose miniature electromechanical energy system", *Artificial Organ*, 18(1), 1994.

4. **Ling J.**, Bovik A.C., "Smoothing low SNR molecular images via anisotropic median-diffusion", *IEEE Transaction on Medical Imaging,* 21(4), pp.377-384, 2002

5. **Ling J.**, Weitman S.D., Miller M.A., Moore R.V., Bovik A.C., "Direct Raman imaging techniques for studying the subcellular distribution of a drug", *Applied Optics*, Vol. 41, No. 28, October 1 2002.

D. Conference Proceedings Publications:

1. **Ling J.**, Takatani S., Noon G.P., Nose Y., "In vivo studies of reflectance pulse oximeter sensor", *Proceedings of the SPIE*, 1993.

2. **Ling J.**, Takatani S., Noon G.P., Nose Y., "In vivo studies of reflectance pulse oximeter sensor", *Abstracts of the 11th Annual Conference on Biomedical Engineering Research in Houston*, 1993 .

3. **Ling J.**, Ktonas P.Y., "Quantitative EEG differences between sleep stage 1 and sleep stage 1-REM", *Proceedings of the Annual Meeting of the American Electro-encephalograph Society*, 1993.

4. Takatani S., Orime Y., Tasai K., Ohara Y., Mizuguchi K., Naito K., Shimono T., Matsuda Y., **Ling J.**, Damm G., Glueck J., Noon G., Nose Y., "A hybrid micro-electronic control driver for totally implantable electromechanical TAH and VAD", *ASAIO Proceedings* 1994. (Abstract)

5. Takatani S., Ohara Y., Jacobs G., Orime Y., Tasai K., **Ling J.**, Glueck J., Noon G., Nose Y., "Roller screw muscle power translation system: design and efficiency analysis", *ASAIO Proceedings* 1994. (Abstract)

6. **Ling J.**, Robey B.L., "A Fuzzy Logic Model for Neural and Hormonal Compensation in Hemorrhagic Shock", *18th Annual International Conference of IEEE Engineering in Medicine and Biology, Amsterdam, The Netherlands,* 1996.

7. **Ling J.**, "A Method to Model Biomedical Systems Using Fussy Logic and Genetic Algorithms", *17th Southern Biomedical Engineering Conference, San Antonio, Texas,* 1998.

8. **Ling J.,** Guerra J.M., Robey B.L., Winter D.C., "Continuous Cardiac Output Determination from Blood Pressure Waveform Using a Fuzzy Logic Model", *21st Annual International Conference of IEEE Engineering in Medicine and Biology, Amsterdam, The Netherlands*, 1999.

9. **Ling J.,** Moore R.V., Miller M, Bovik, A.C., Weitman S.D., "Application of Raman Imaging Microscopy to Evaluate Drug Distribution within Cancer Cells", *91st Annual Meeting of American Association for Cancer Research, San Francisco, California*, 2000.

10. **Ling J.,** Bovik A.C., "Modeling and Restoration of Raman Microscopic Images", *IEEE 2000 International Conference on Image Processing, Vancouver, Canada*, 2000.

11. **Ling J.,** Miller M.A., Cruz E., Weitman S.D., Bovik A.C., "Monitoring the Temporal Distribution of Taxol® in a Living Tumor Cell Using Raman Imaging Microscopy" *American Association for Cancer Research Special Conference: Molecular Imaging In Cancer, Naples, Florida,* January, 2002.

12. **Ling J.**, Miller M.A., Cruz E., Weitman S.D., "Cellular Level Drug Mechanism Study Using Raman Imaging" *6th Annual Conference & Exhibition on Drug Discovery Technology*, Stuttgart, Germany, April, 2002.

13. **Ling, J.**, Daniel D.P., Moravis, D.E., Miller, M.A., "Using Raman Imaging to Map Localized Stress Around Osteocyte Lacuna", the Federation of Analytical Chemistry and Spectroscopy Societies Conference 2003, Florida, October, 2003.

Patents:

1. U.S. Patent No. 6007491
   Cardiac output monitor using fuzzy logic blood pressure analysis

2. U.S. Patent Pending
   Virtual Reality Sustem Locomotion Interface Utilizing a Pressure-Sensing Mat

3. U.S. Patent Pending
   Methodology of Using Raman Imaging Microscopy for Evaluating Drug Action within Living Cells

MICHAEL A. MILLER
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Materials Characterization and Development Section
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B.S. in Chemistry, University of Texas at San Antonio, 1983
M.S. in Physical Chemistry, University of Texas at San Antonio, 1992

Mr. Miller has conducted extensive research in developing analytical methods and theoretical models aimed at determining or predicting the kinetic and structural disposition of organic, inorganic, and polymeric compounds in complex systems using advanced instrumentation (HPLC, GC, SFC, and gel chromatography, high field NMR, fiber-optic Raman, electrochemical impedance spectroscopy and mass spectrometry), sensors, and atomistic modeling. In the field of theoretical chemistry, he co-developed a quantum-mechanical model to simulate interactions of molecular ion beams on metal and semi-conducting surfaces. Also, he developed a classical, molecular dynamics model to study surface relaxations in pure metals. As principal or co-investigator, he has contributed to numerous and diverse projects in applied physical chemistry, ranging in topics from process development for the integration of highly ordered polymeric structures in polyolefins and structural plastics, to the implementation of fiber-optic Raman spectroscopy in conjunction with kinetic and transport models for the intelligent processing of composite materials. His R&D areas of expertise include modeling of physicochemical processes, the study of surface interfaces as applied to chemical systems, the development and application of analytical methods, imaging, and sensors to study the fate of molecules/polymers in complex systems, and the chemical-state-based intelligent processing of materials.

PROFESSIONAL CHRONOLOGY: Technician, Florida Solar Energy Research Center, Cape Canaveral, Florida, 1979-80; research associate, University of Texas Health Science Center at San Antonio, 1982-85; Graduate Student Fellow, University of Houston-Photoelectron Spectroscopy Laboratory, 1983; Southwest Research Institute, 1985-(research scientist, 1985-91; Senior Research Scientist, department of Applied Chemistry and Chemical Engineering, 1991-95; Senior Research Scientist, Materials Development Section, Mechanical and Materials Engineering Division, 1995-2000; Manager, Materials Development Section, Mechanical and Materials Engineering Division, 2000-).

June 2001

SELECTED LIST OF PUBLICATIONS:

Modeling and Simulation

P. Hochmann, M. A. Miller, and J. W. Rabalais. Reactions of Diatomic Ions with Surfaces. III. Model for Interactions of Heteronuclear Cation Beams with Elemental Surfaces. *J. Phys. Chem.* 89, 2751, **1985.**

M. A. Miller and R. S. Geary. RIA-Linked Microdialysis Sampling in the Awake Rat: Application to Free-Drug Pharmacokinetics of Hydrocortisone. *J. Pharm. and Biomed. Anal., 9*(10-12), 901-910, **1991.**

Michael A. Miller and John F. Maguire. A Cumulant Expansion Approach to Predict the Service Lifetime of Polymers and Polymer Composites. *ASME*, DE-Vol. 87, 63-66, **1995.**

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S. J. Hudak, Jr., M. A. Miller, G. Cragnolino, and D. Dunn (Southwest Research Institute), Y. F. Li, J. Wang, C. Laird, and J. DeLuccia (University of Pennsylvania), J. Goodman (Tensiodyn Scientific Corp.), Development of an Electrochemical Sensor for Early Detection of Fatigue Damage in Aircraft, *1997 USAF Aircraft Structural Integrity Program Conference,* December 3, 1997, San Antonio, TX.

S.T. Green, D.M. Deffenbaugh, and M.A. Miller, Trade Study of Five In-Situ Propellant Production Systems for Mars Sample Return Mission, *AIAA Houston Section Technical Symposium*, May 28, 1998, Houston, TX.

Paul, P.P.; Miller, M.A.; Heimrich, M.J.; Schwab, S.T., Emission Control Catalyst Derived from Mesoporous Molecular Sieves. *Proceedings of the Materials Research Society, Microporous and Macroporous Materials*, 431, 117-121, **1996.**

Paul, P.P.; Heimrich, M.J.; Miller, M.A.; Schwab, S.T., Development of a Lean-NO_x Catalyst Containing Metal-Ligand Complex Impregnated Molecular Sieves. *SAE International, Fall Fuels and Lubricants Meeting,* **1996**, Paper # 962050.

Paul, P.P.; Heimrich, M.J.; Miller, M.A., Lean-NO_x Reduction Catalysis by Metal-Complex Impregnated Molecular Sieves – Effect of Ligands and Metals. *Catalysis Today*, 42, 61-71, **1998.**

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Chemical Spectroscopy and Raman Applications

"Overview of Spectroscopic Methods in New Sensor Development", by John F. Maguire and Michael A. Miller, Moderators Review in Optical Sensors for On-line Control, American Society for Non Destructive Testing, Advanced School on Sensors for Process Monitoring and Quality Control, Banff, Canada, June **1995.**

John F. Maguire, Michael A. Miller, Sanjeev Venkatesan, David L. Littlefield, and Steven R. LeClair. An Intelligent Real-Time System for Polymer and Composite Processing. *International Federation of*

Automatic Control (IFAC) and International Association for Mathematics and Computers in Simulation (IMACS. Workshop on Artificial Intelligence in Real-Time Control. J. Kocijian and R. Karba, eds. Bled, Slovenia (November 1995):306.

John F. Maguire, Michael A. Miller, Sanjeev Venkatesan, David Littlefield and Steven R. LeClair, An Intelligent Real-Time System for Polymer and Composite Processing, *Control Engineering and Practice*, accepted for publication, March **1996**.

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